

FIG. 1

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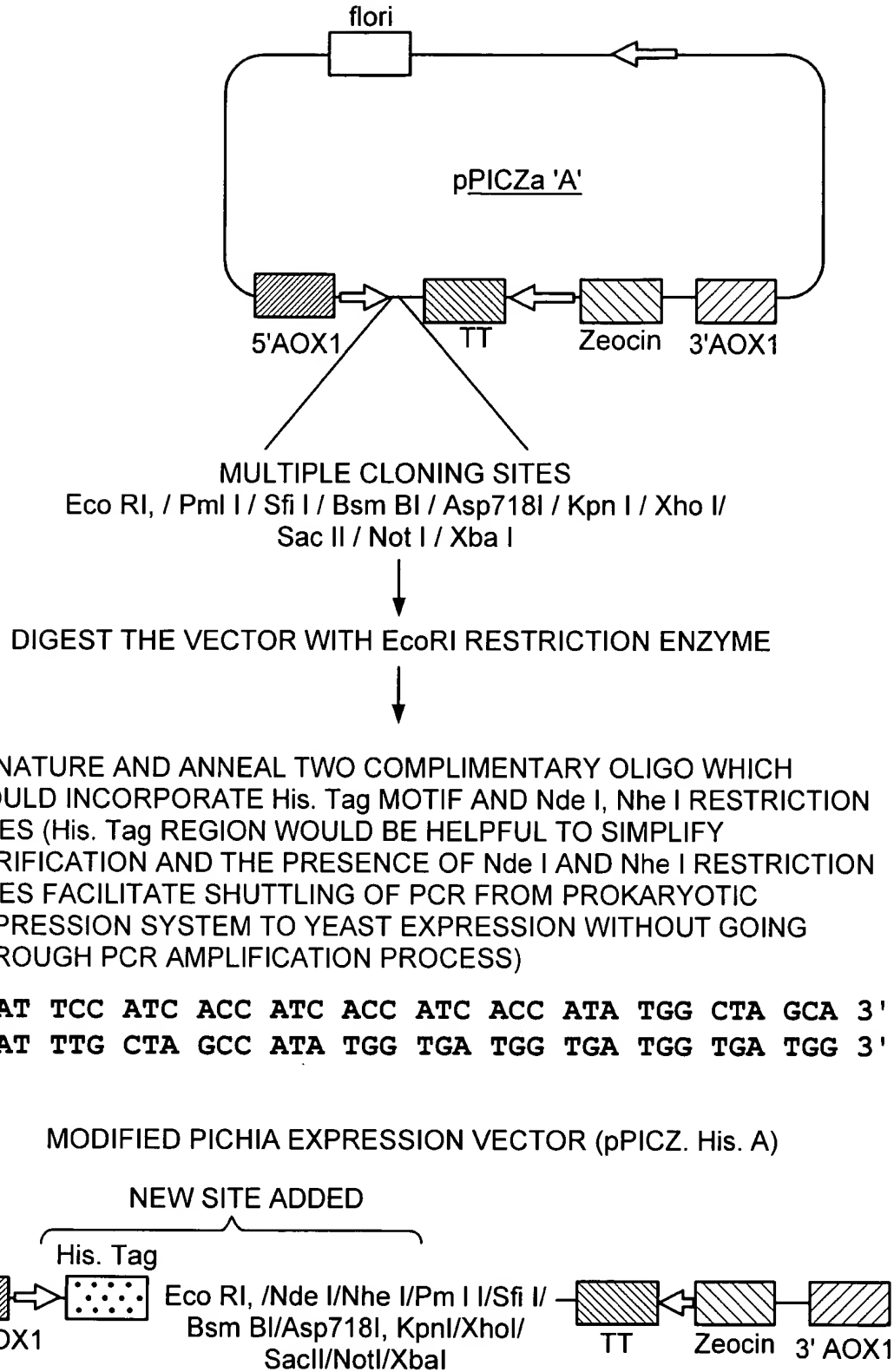


FIG. 2

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FLOW CHART: CLONING OF ENDOSTATIN (MOUSE)  
 INTO PICHIA EXPRESSION SYSTEM

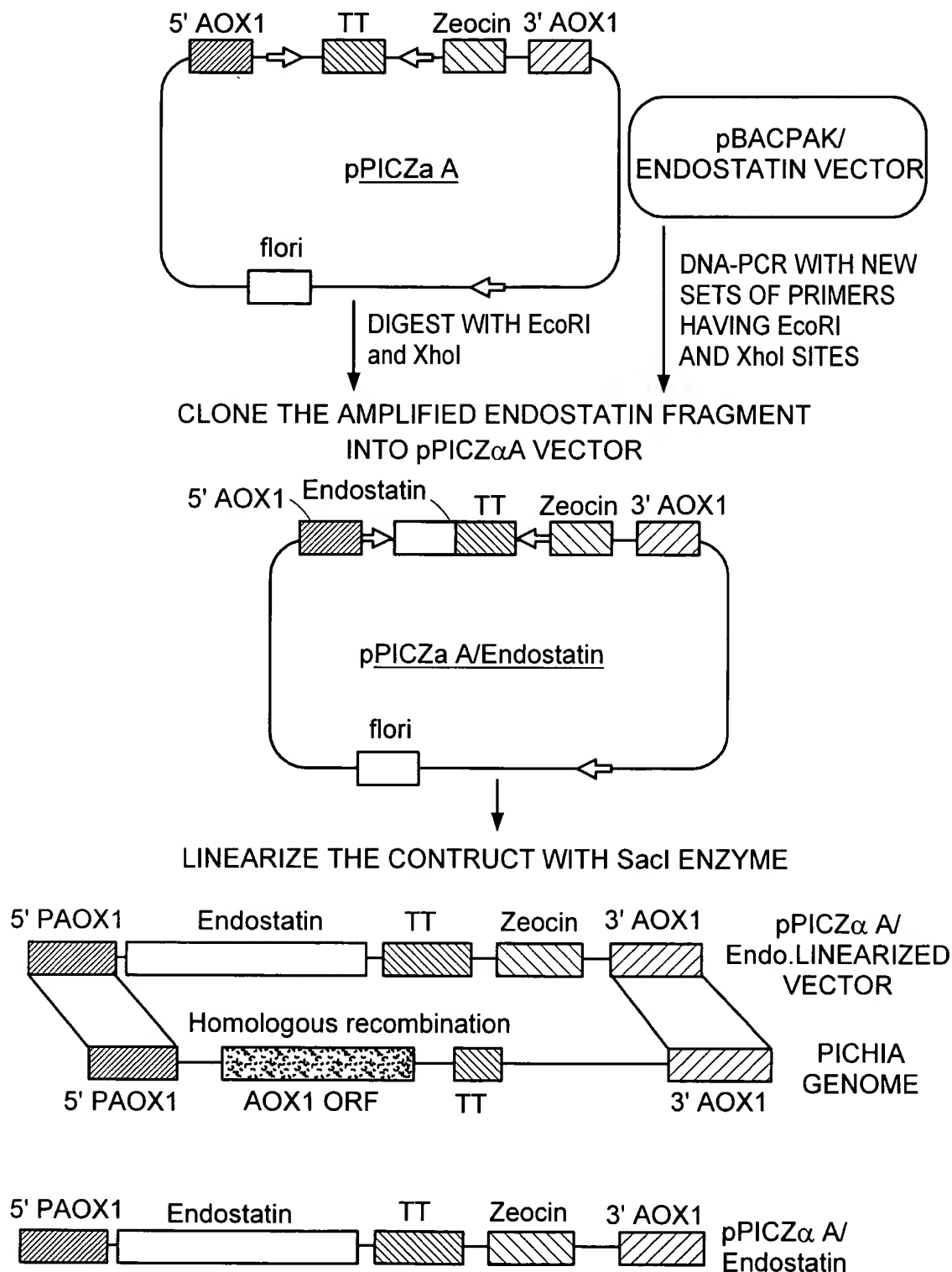


FIG. 3



FIG. 4A
FIG. 4B

FIG. 4

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endo sequence from Collagen XVIII.

Sequence Range: 1-555

Nucleotide 1 = Start for Endostatin and fragments EM1 and EM2.

EM1 fragment ends at nucleotide 525, EM2 fragment ends at nucleotide 501.

5	10	15	20	25	30	35	40	45							
CAT	ACT	CAG	GAC	TTT	CAG	CCA	GTG	CTC	CAC	CTG	GTG	GCA	CTG	AAC	
GTA	TGA	GTA	GTC	CTG	AAA	GTC	GGT	CAC	GAG	GTG	GAC	CAC	CGT	GAC	TTG
50	55	60	65	70	75	80	85	90	95						
ACC	CCC	CTG	TCT	GGA	GGC	ATG	CGT	GGT	ATC	CGT	GGA	GCA	GAT	TTC	CAG
TGG	GGG	GAC	AGA	CCT	CCG	TAC	GCA	CCA	TAG	GCA	CCT	CGT	CTA	AAG	GTC
100	105	110	115	120	125	130	135	140							
TGC	TTC	CAG	CAA	GCC	CGA	GCC	GTG	GGG	CTG	TCG	GGC	ACC	TTC	CGG	GCT
ACG	AAG	GTC	GTT	CGG	GCT	CGG	CAC	CCC	GAC	AGC	CCG	TGG	AAG	GCC	CGA
145	150	155	160	165	170	175	180	185	190						
TTC	CTG	TCC	TCT	AGG	CTG	CAG	GAT	CTC	TAT	AGC	ATC	GTG	CGC	CGT	GCT
AAG	GAC	AGG	AGA	TCC	GAC	GTC	CTA	GAG	ATA	TCG	TAG	CAC	GCG	GCA	CGA
195	200	205	210	215	220	225	230	235	240						
GAC	CGG	GGG	TCT	GTG	CCC	ATC	GTC	AAC	CTG	AAG	GAC	GAG	GTG	CTA	TCT
CTG	GCC	CCC	AGA	CAC	GGG	TAG	CAG	TTG	GAC	TTC	CTG	CTC	CAC	GAT	AGA

FIG. 4A



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245 250 255 260 265 270 275 280 285  
CCC AGC TGG GAC TCC CTG TTT TCT GGC TCC CAG GGT CAA CTG CAA CCC  
GGG TCG ACC CTG AGG GAC AAA AGA CCG AGG GTC CCA GTT GAC GTT GGG  
290 295 300 305 310 315 320 325 330 335  
GGG GCC CGC ATC TTT TCT TTT GAC GGC AGA GAT GTC CTG AGA CAC CCA  
CCC CGG GCG TAG AAA AGA AAA CTG CCG TCT CTA CAG GAC TCT GTG GGT  
340 345 350 355 360 365 370 375 380  
GCC TGG CCG CAG AAG AGC GTA TGG CAC GGC TCG GAC CCC AGT GGG CGG  
CGG ACC GGC GTC TTC TCG CAT ACC GTG CCG AGC CTG GGG TCA CCC GCC  
385 390 395 400 405 410 415 420 425 430  
AGG CTG ATG GAG AGT TAC TGT GAG ACA TGG CGA ACT GAA ACT ACT GGG  
TCC GAC TAC CTC TCA ATG ACA CTC TGT ACC GCT TGA CTT TGA TGA CCC  
435 440 445 450 455 460 465 470 475 480  
GCT ACA GGT CAG GCC TCC TCC CTG CTG TCA GGC AGG CTC CTG GAA CAG  
CGA TGT CCA GTC CCG AGG AGG GAC GAC AGT CCG TCC GAG GAC CTT GTC  
485 490 495 500 505 510 515 520 525  
AAA GCT GCG AGC TGC CAC AAC AGC TAC ATC GTC CTG TGC ATT GAG AAT  
TTT CGA CGC TCG ACG GTG TTG TCG ATG TAG CAG GAC ACG TAA CTC TTA  
530 535 540 545 550 555  
AGC TTC ATG ACC TCT TTC TCC AAA TAG  
TCG AAG TAC TGG AGA AAG AGG TTT ATC

FIG. 4B



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# ENDOSTATIN

Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
MOUSE ENDOSTATIN				
pET17b/ his.mendo	5'-GGC ATA TGC ATA CTC ATC AGG- ACT TT-3' (up) (SEQ ID NO:4)	NdeI & XhoI	Prokaryotic expression, pET ( <i>E. coli his.endo</i> )	MGHHHHHHHHSSGHIDDDDKH M-mendo (SEQ ID NO:14)
	5' AAC TCG AGC TAT TTG GAG AAA- GAG GT-3' (down) (SEQ ID NO:5)			
pET28a/ mendo	5'-GGC ATA TGC ATA CTC ATC AGG- ACT TT-3' (up) (SEQ ID NO:4)	NdeI & NotI	Prokaryotic expression, pET ( <i>E. coli his.endo</i> )	MGSSHHHHHHSSGLVPRGSHM- mendo (SEQ ID NO:15)
	5'-AAG CGG CCG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)			
pET28a/ EM-1	5' TTC CAT ATG CAT ACT CAT CAG- GAC TTT CAG CCA-3' (up) (SEQ ID NO:7)		Prokaryotic expression, pET ( <i>E. coli EM1</i> )	MGSSHHHHHHSSGLVPRGSHM-me ndo (SEQ ID NO:15)
	5' TTA GCG GCC GCC TAC TCA ATG- CAC AGG ACG ATG TA-3' (down) (SEQ ID NO:8)			
pET28a/ EM-2	5' TTC CAT ATG CAT ACT CAT CAG- GAC TTT CAG CCA-3' (up) (SEQ ID NO:7)		Prokaryotic expression, pET ( <i>E. coli EM2</i> )	MGSSHHHHHHSSGLVPRGSHM-me ndo (SEQ ID NO:15)
	5' TTA GCG GCC GCC TAG TTG TGG- CAG CTC GCA GCT TTC TG-3' (down) (SEQ ID NO:9)			

FIG. 5A

Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
pPICZαA/ mendo <i>(yeast mus endo)</i>	5' GGG AAT TCC ATA CTC ATC AGG- ACT TT-3' (up) (SEQ ID NO:10)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZαA <i>(yeast mouse endostatin)</i>	EF-mendo (SEQ ID NO:16)
	5' AAG CGG CCG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)			
pPICZαA/ His.mendo	5' AAG AAT TCC ATC ATC ATC ATC- ATC ACA GCA GC-3' (up) (SEQ ID NO:11)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZαA <i>(yeast mouse his.endostatin)</i>	EFMGHHHHHHHHSSGHIDDDD KHM-mendo (SEQ ID NO:17)
	5' AAG CGG CCG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)			
HUMAN ENDOSTATIN				
pPICZαA/ Hendo	5' TTT GAA TTC GCC CAC AGC CAC- CGC GAC TTC CAG CCG GTG CTC- CAC-3' (up) (SEQ ID NO:12)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZαA <i>(yeast human endostatin)</i>	EF-hendo (SEQ ID NO:18)
	5' AAA AGC GGC CGC CTA CTT GGA- GGC AGT CAT GAA GCT GTT CTC- AAT-3' (down) (SEQ ID NO:13)			

FIG. 5B

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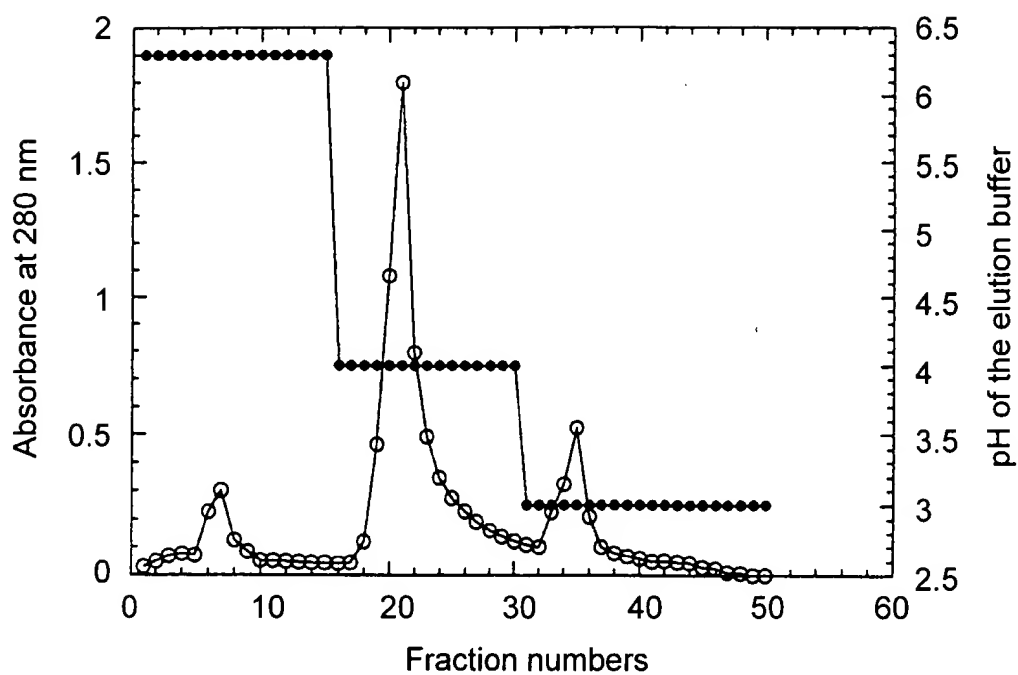


FIG. 6A

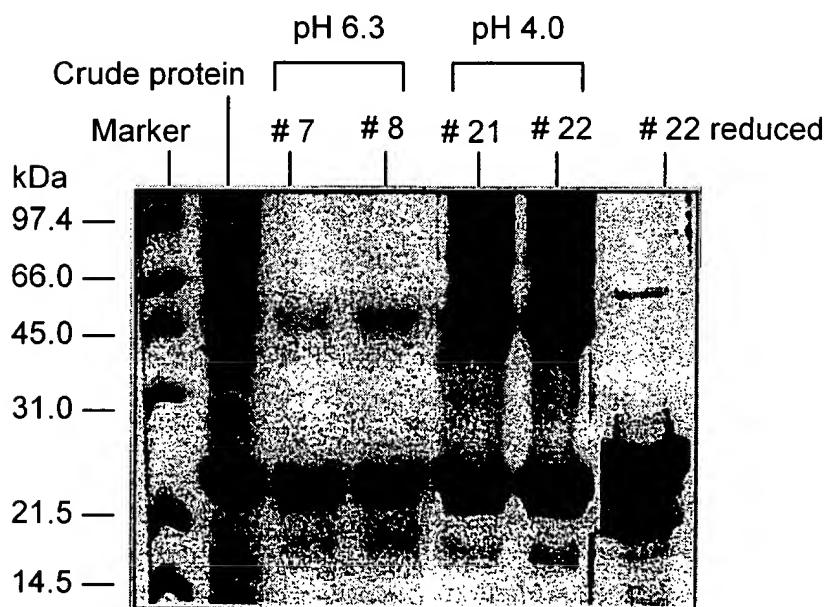


FIG. 6B



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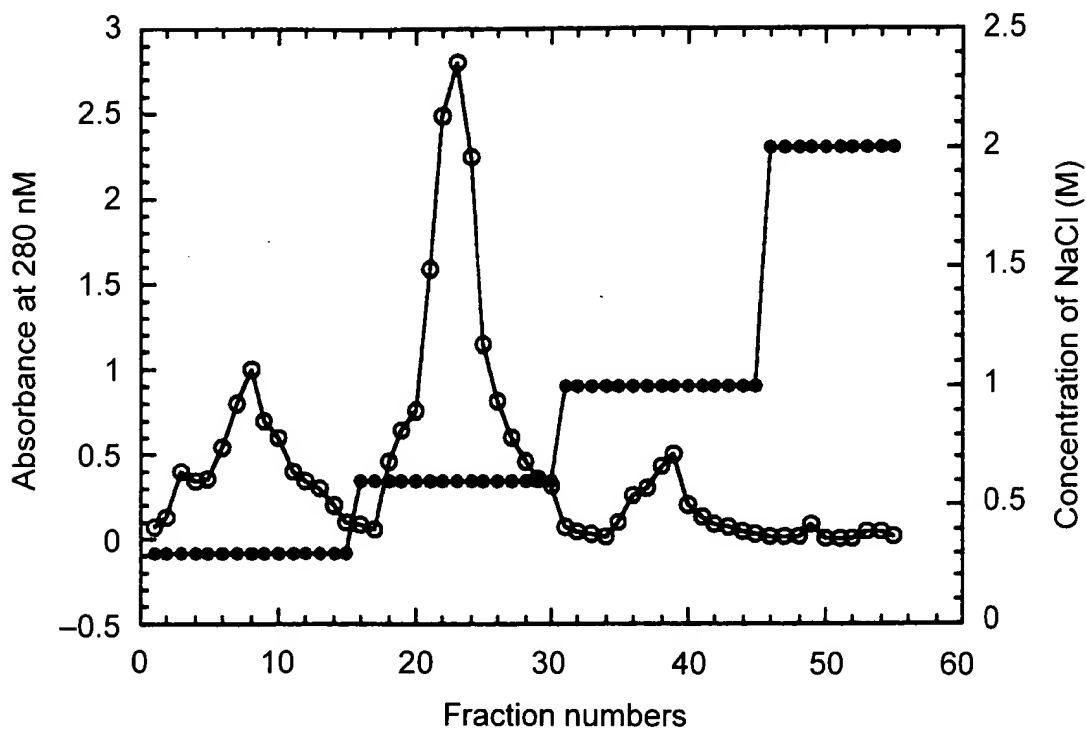


FIG. 7A

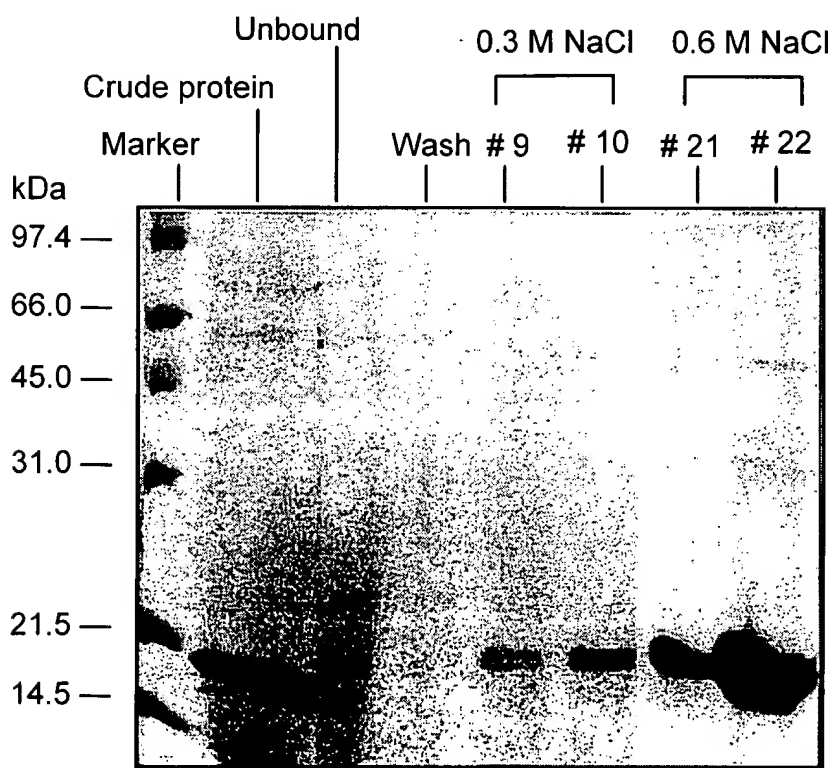


FIG. 7B

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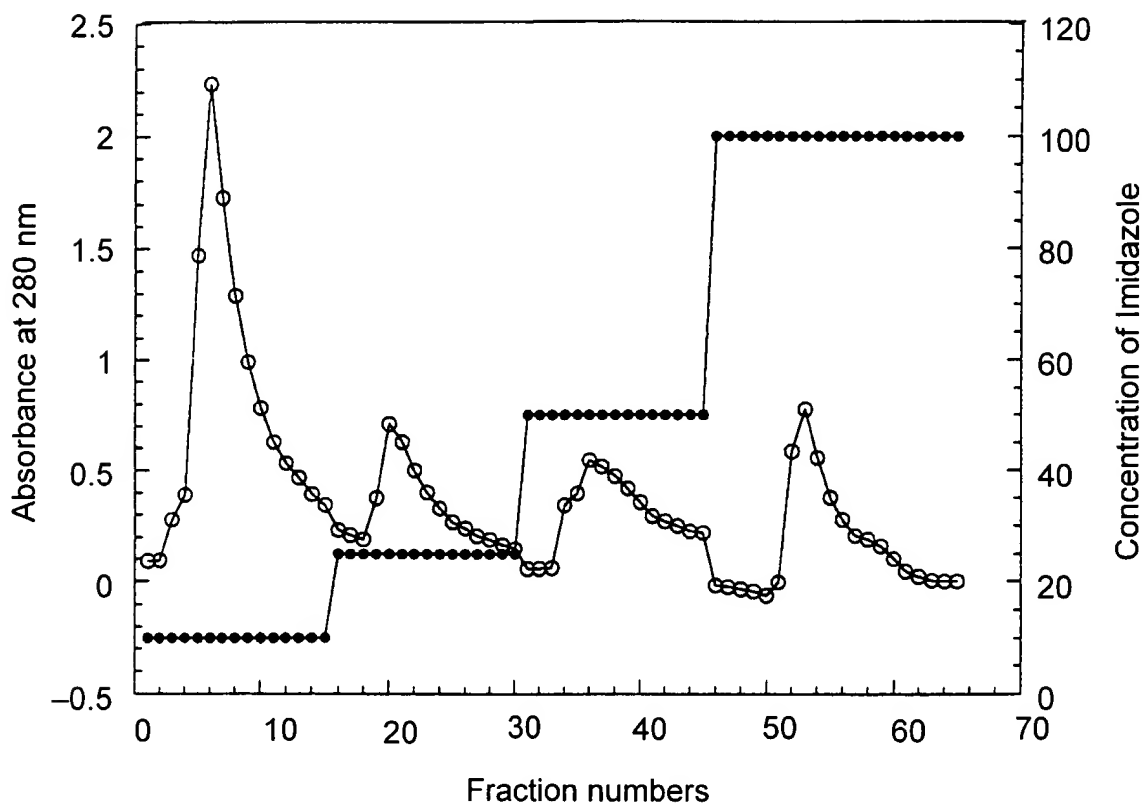


FIG. 8A

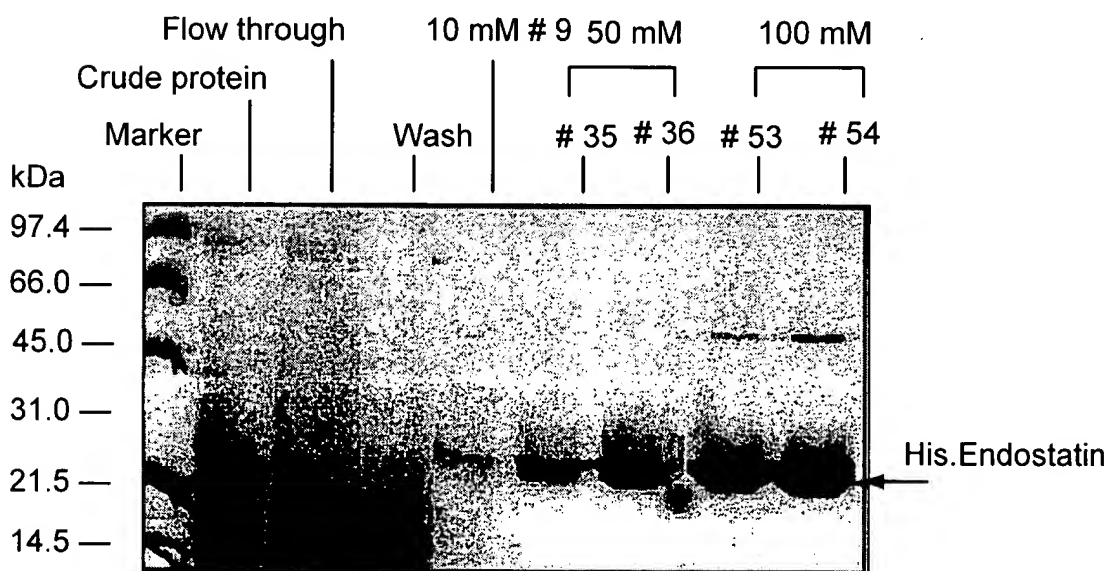
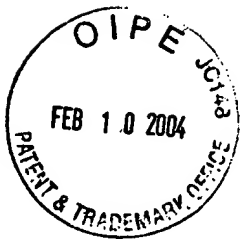


FIG. 8B



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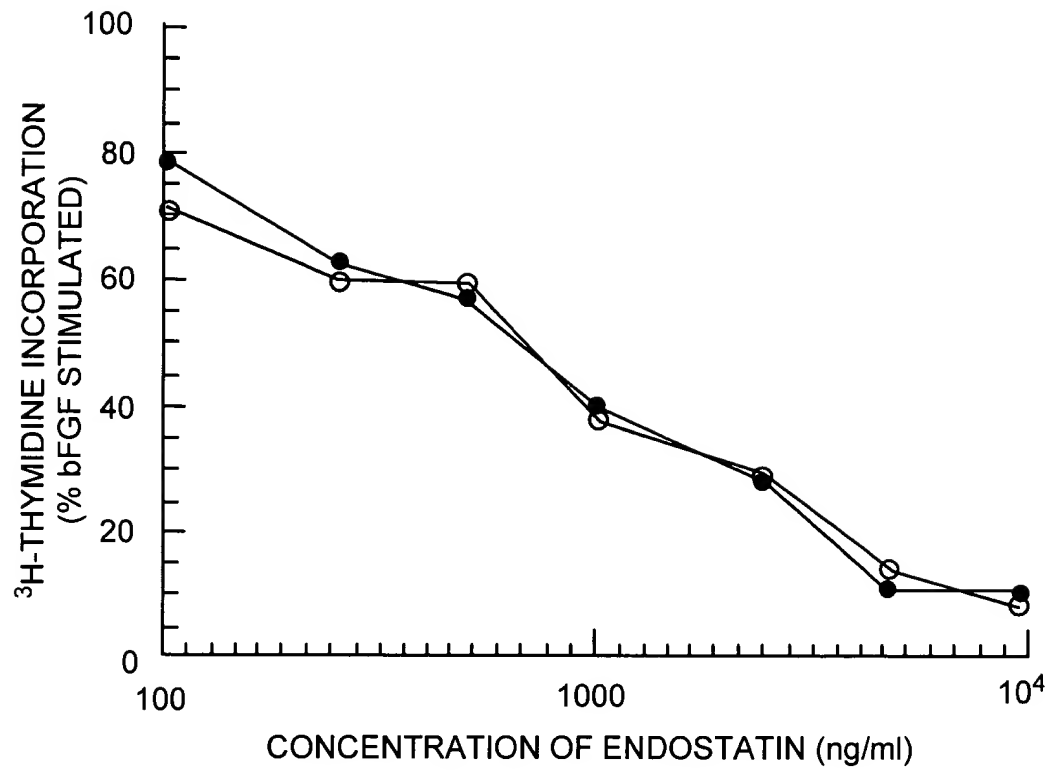


FIG. 9

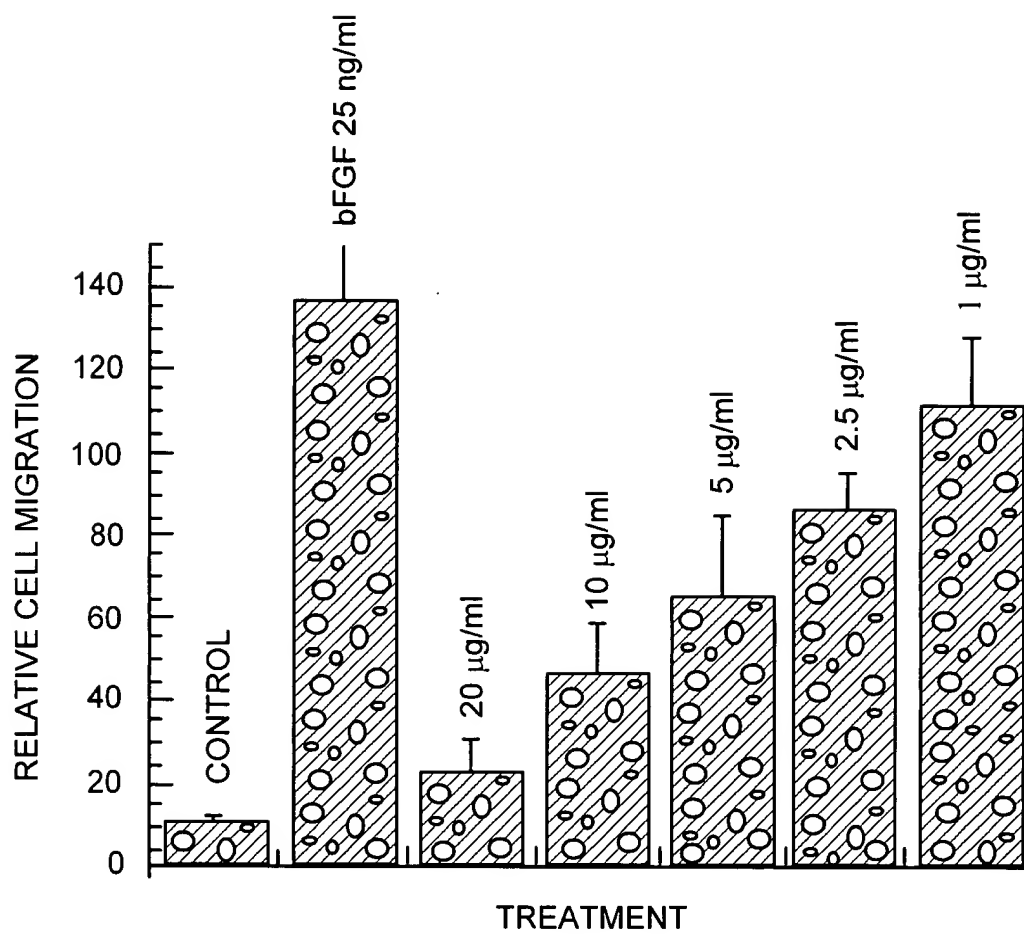


FIG. 10

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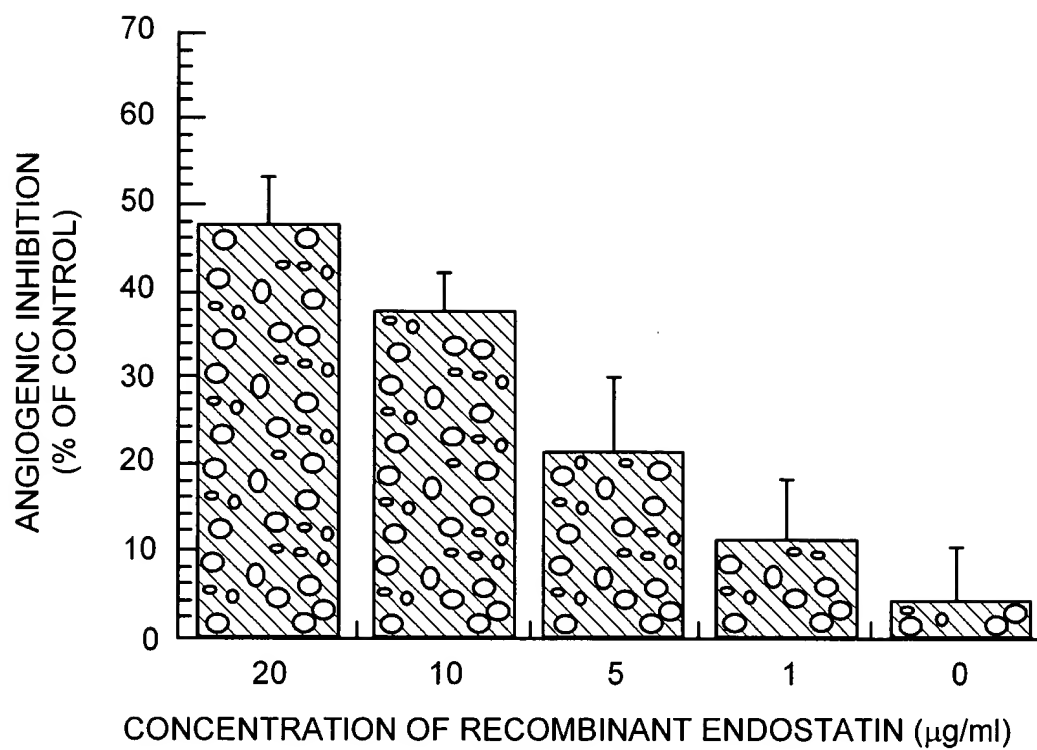


FIG. 11A

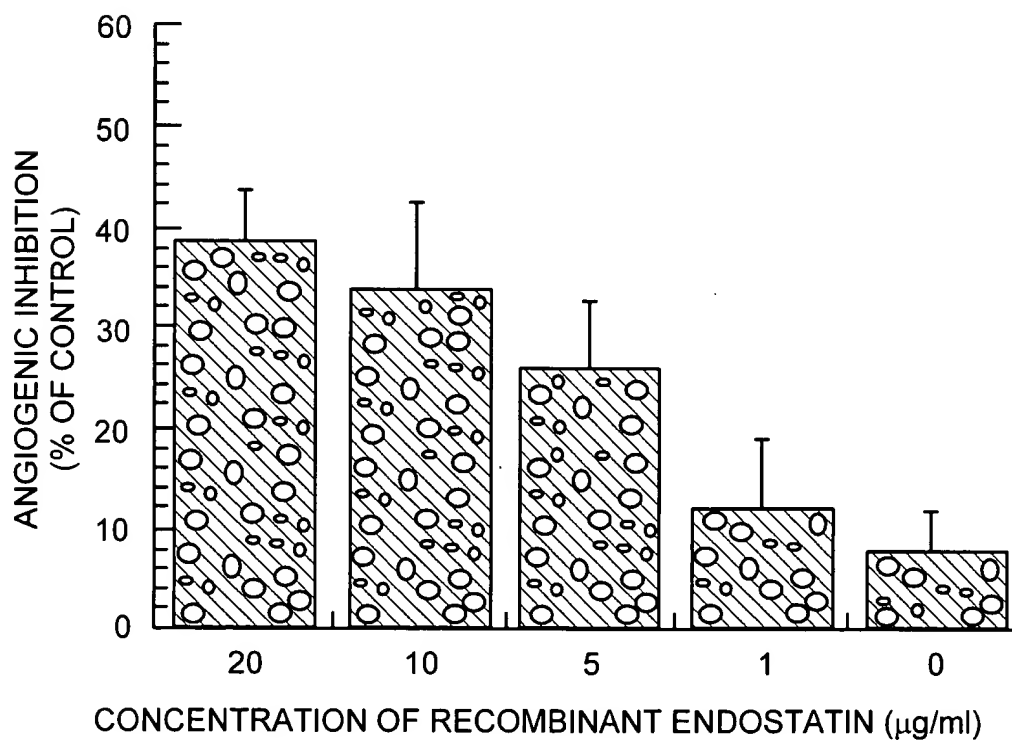


FIG. 11B

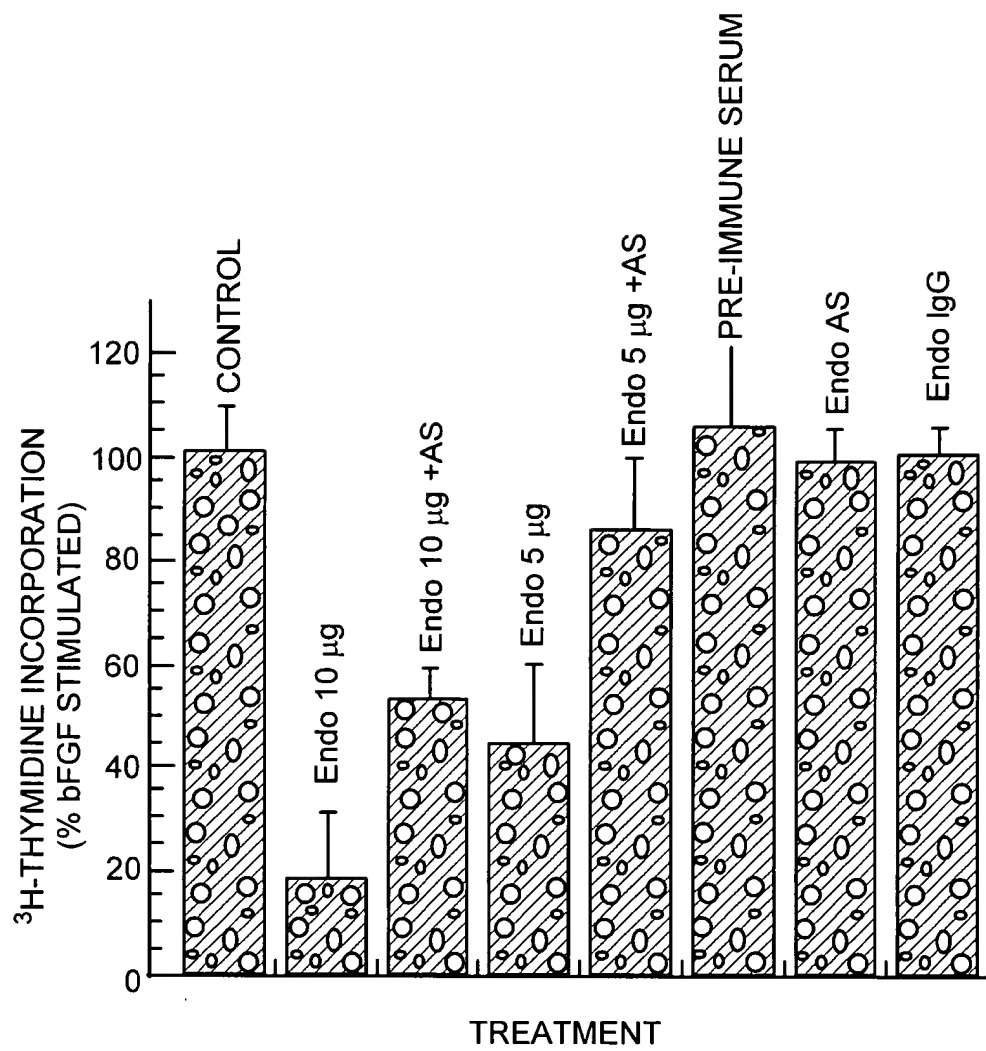


FIG. 12



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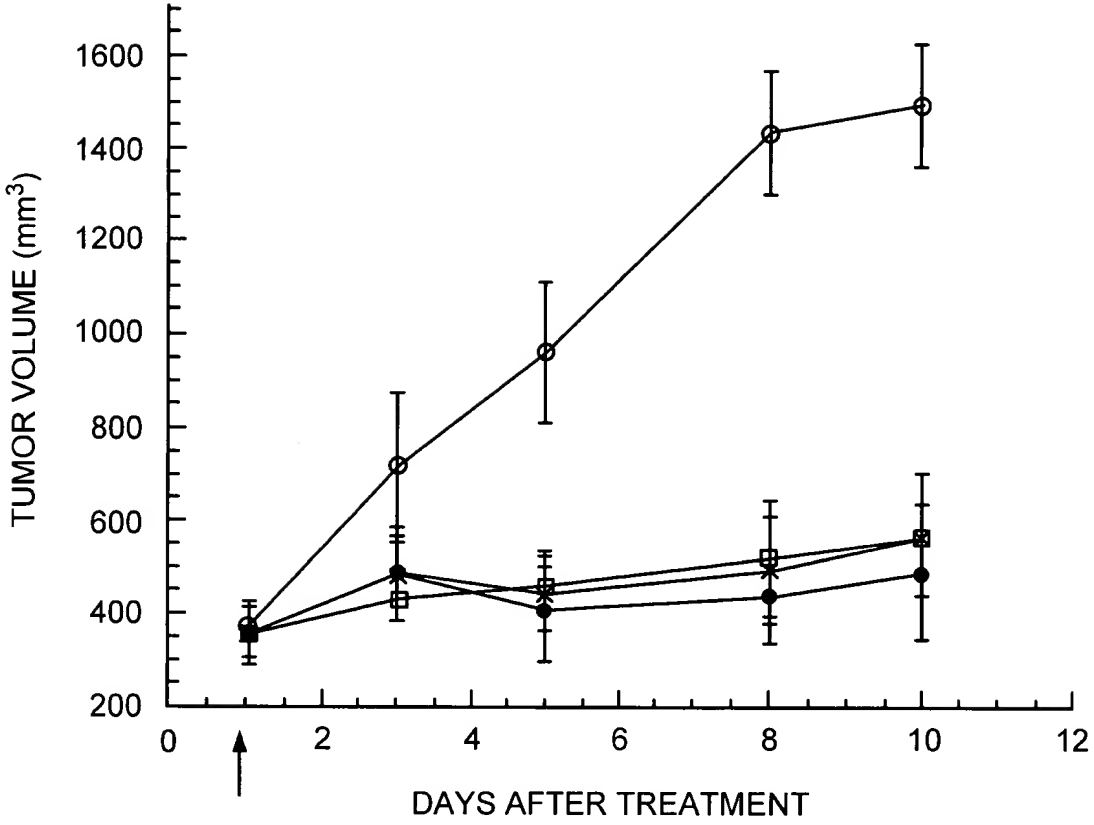


FIG. 13A

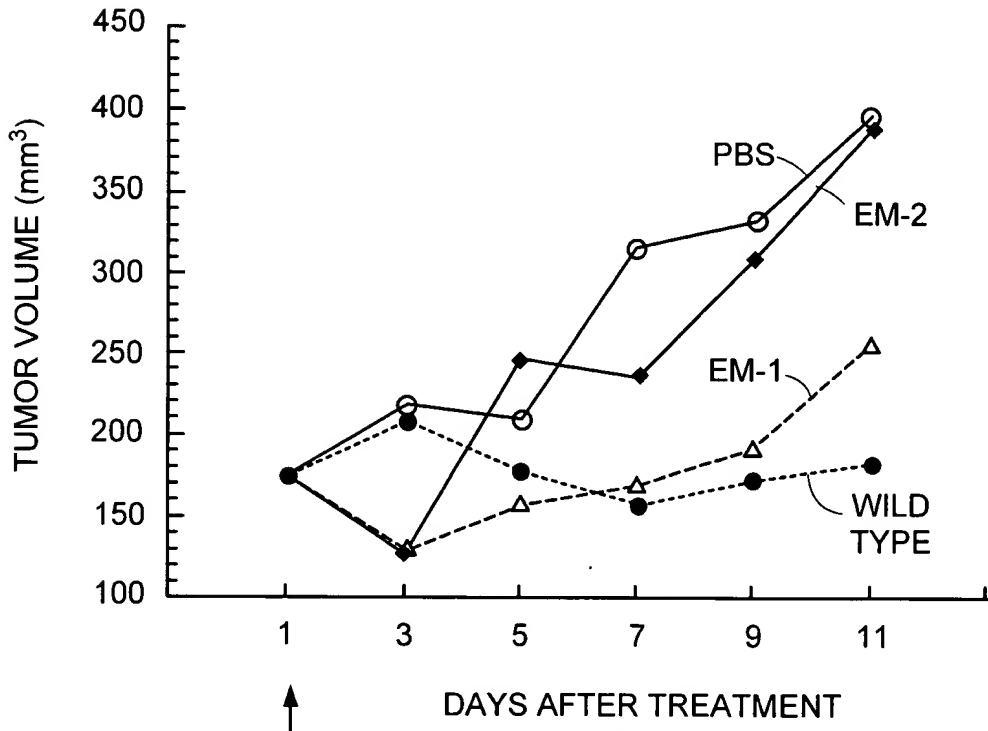


FIG. 13B

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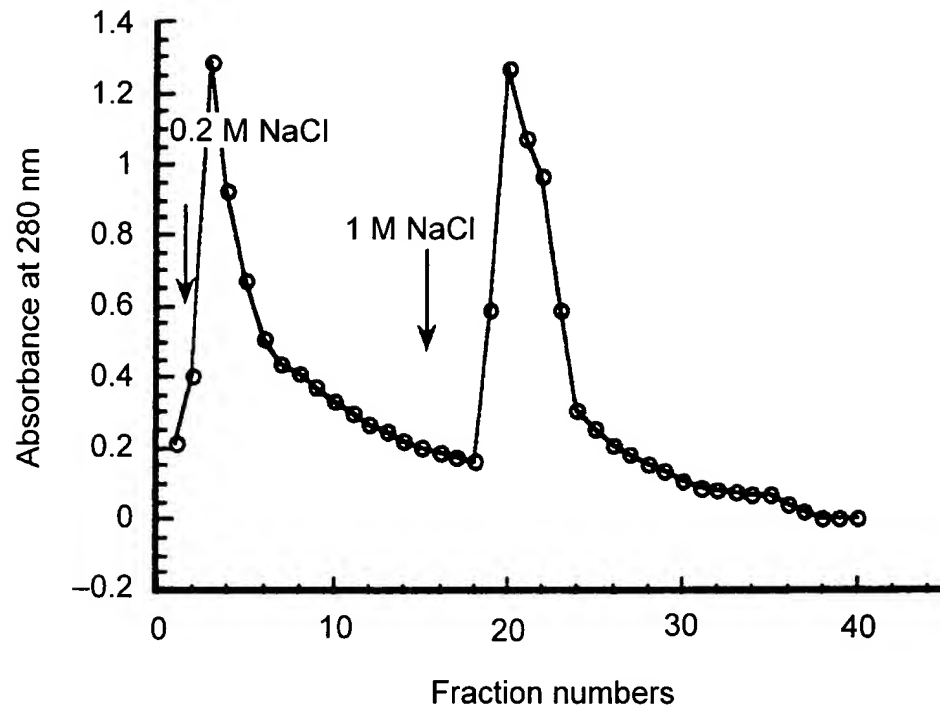


FIG. 14A

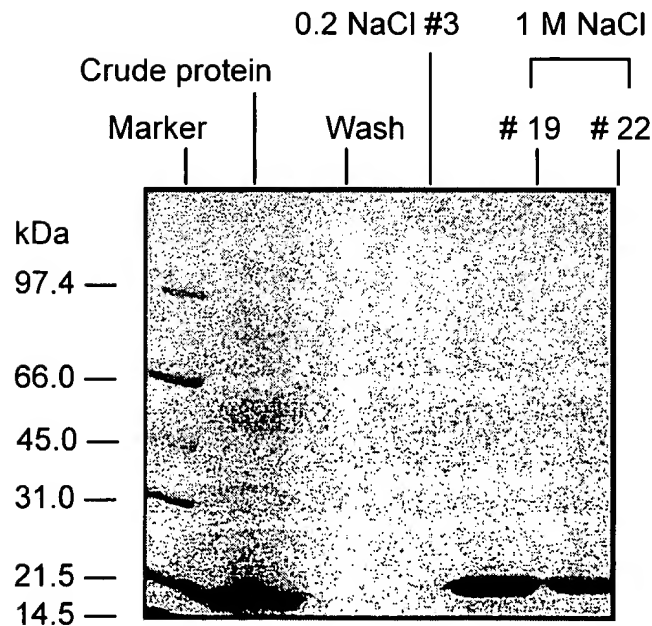


FIG. 14B



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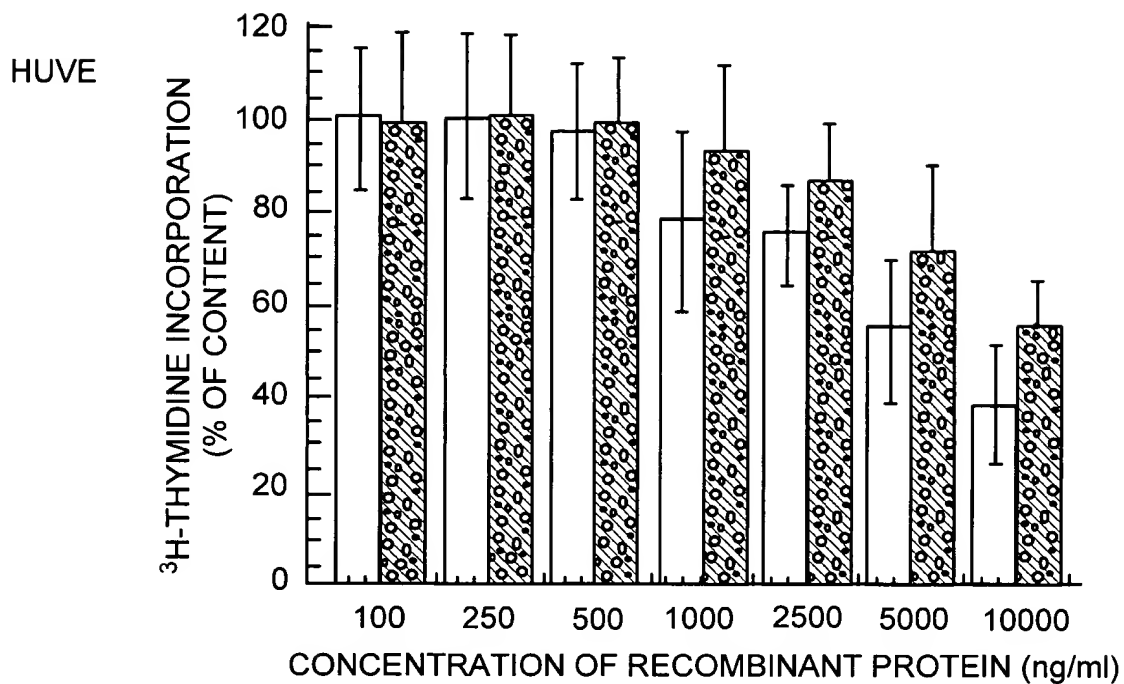


FIG. 15A

HMEV-L

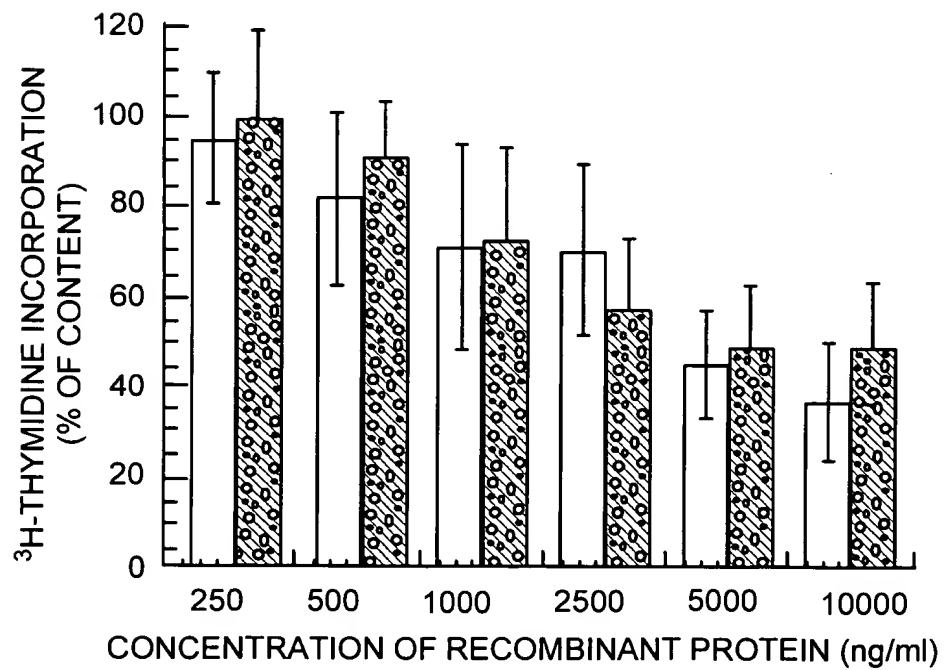


FIG. 15B

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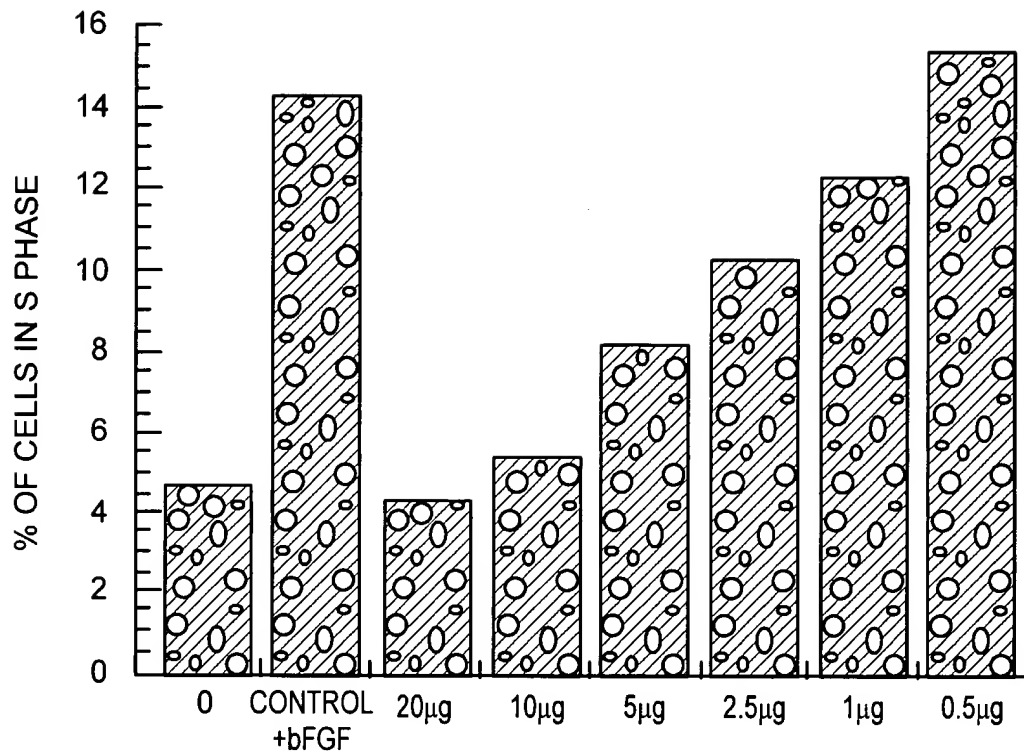


FIG. 16A

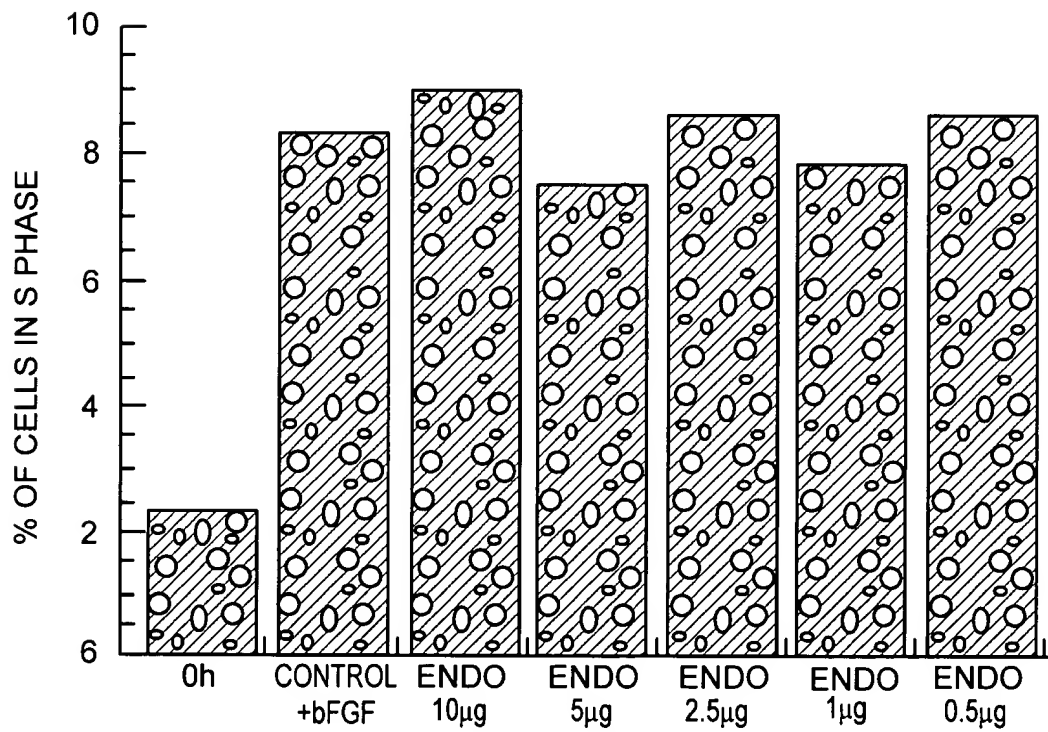


FIG. 16B

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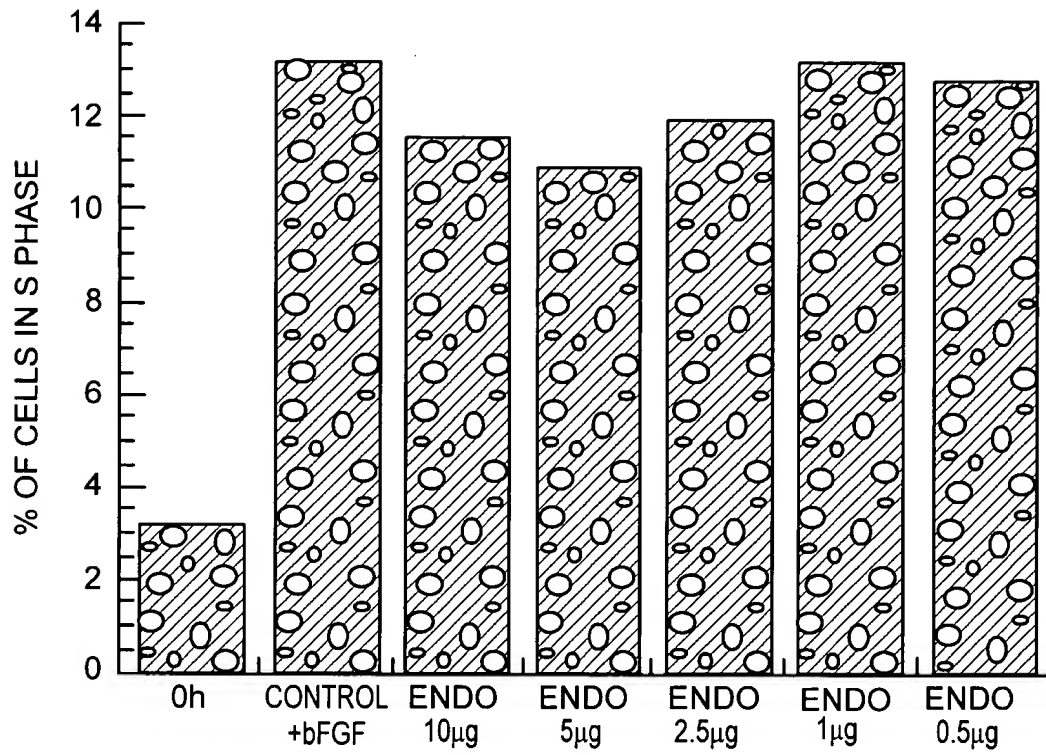


FIG. 16C

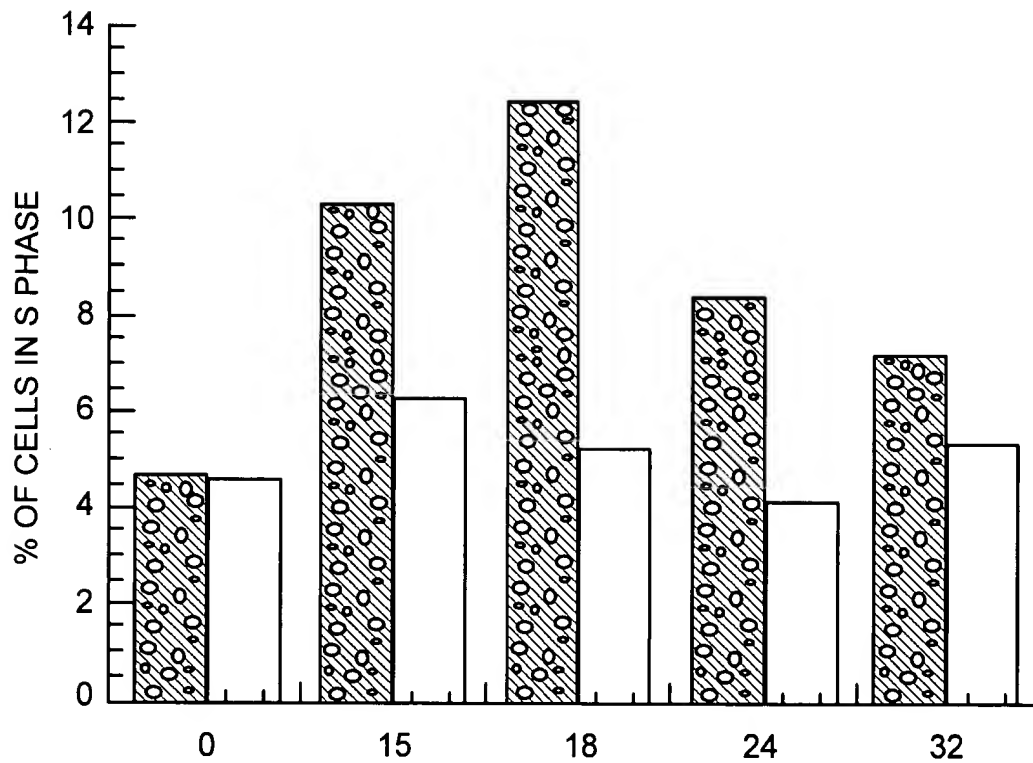


FIG. 16D

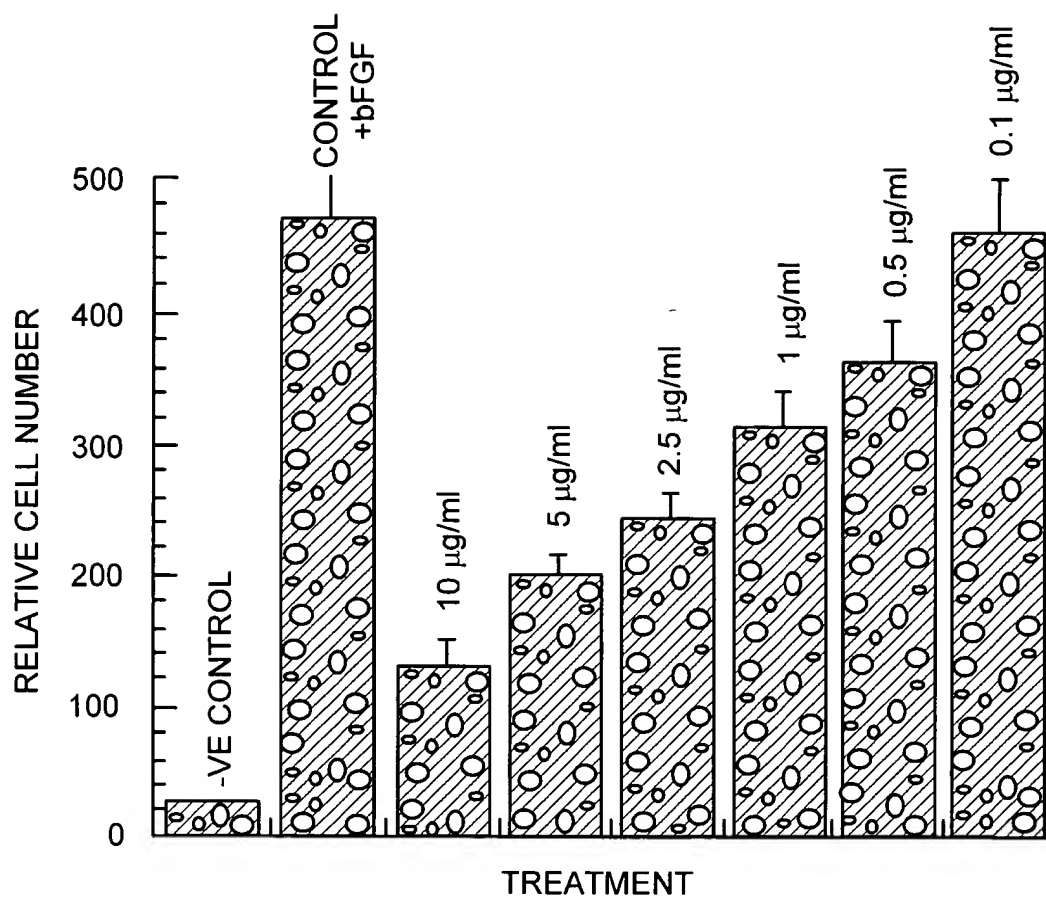


FIG. 17

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Purification of Angiostatin (mouse): Lysine Sepharose 4B column

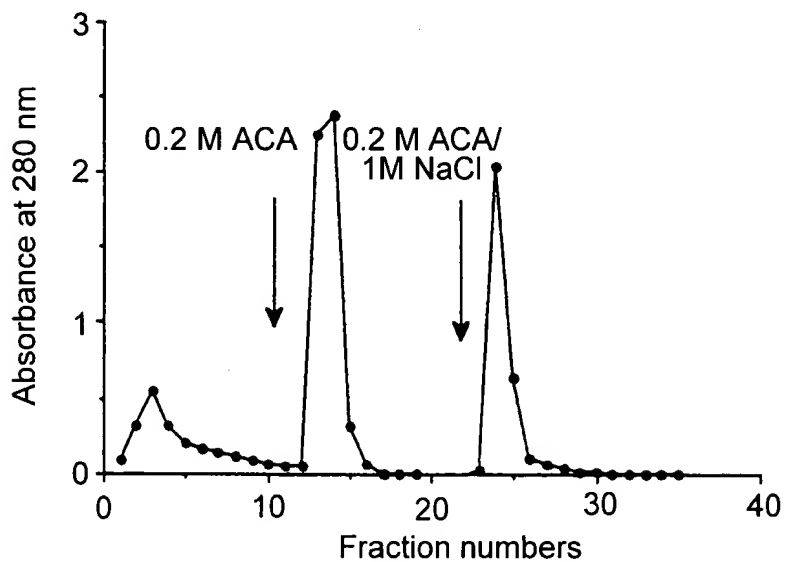


FIG. 18A

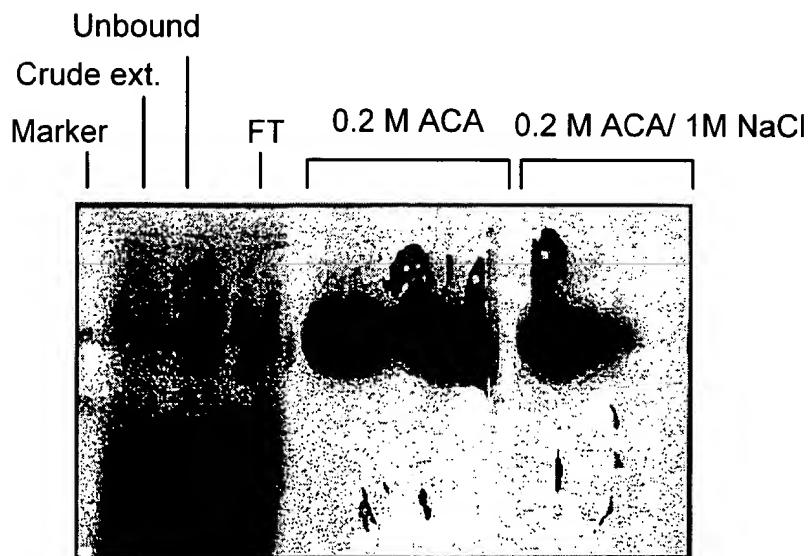


FIG. 18B



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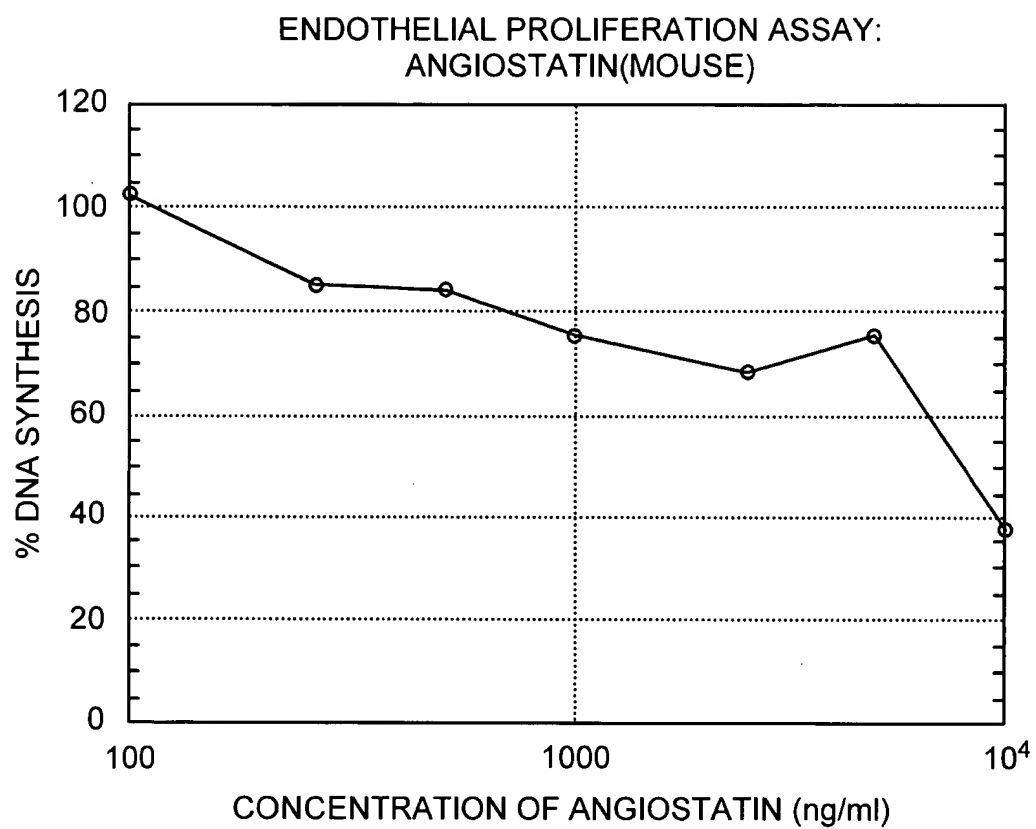


FIG. 19



EFFECT OF RECOMBINANT EDOSTATIN (MOUSE) ON TUMOR GROWTH  
(RENAL CANCER CELL LINE 786-0)

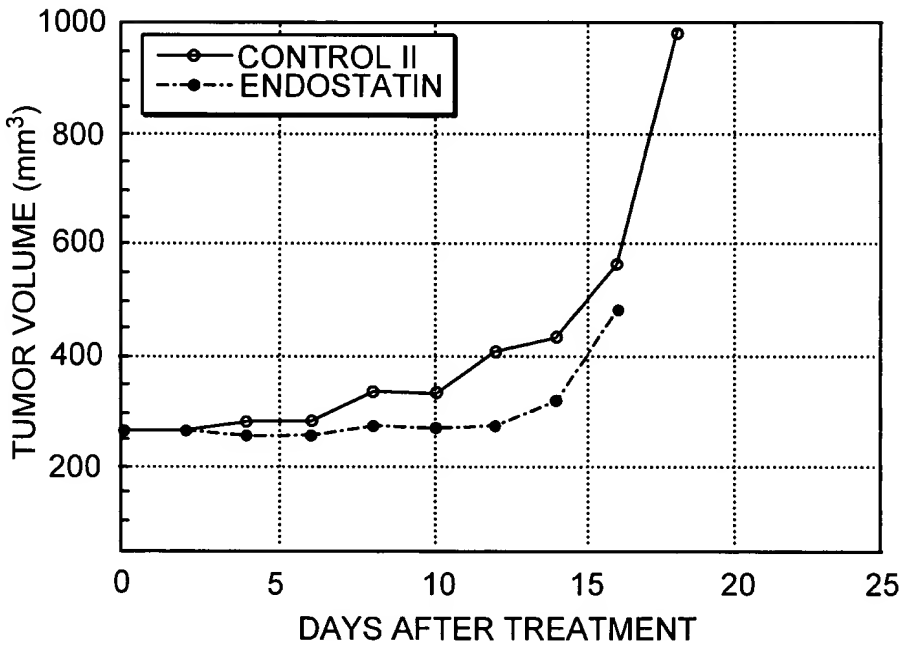


FIG. 20

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Purification of Angiostatin (Human): Lysine Sepharose column

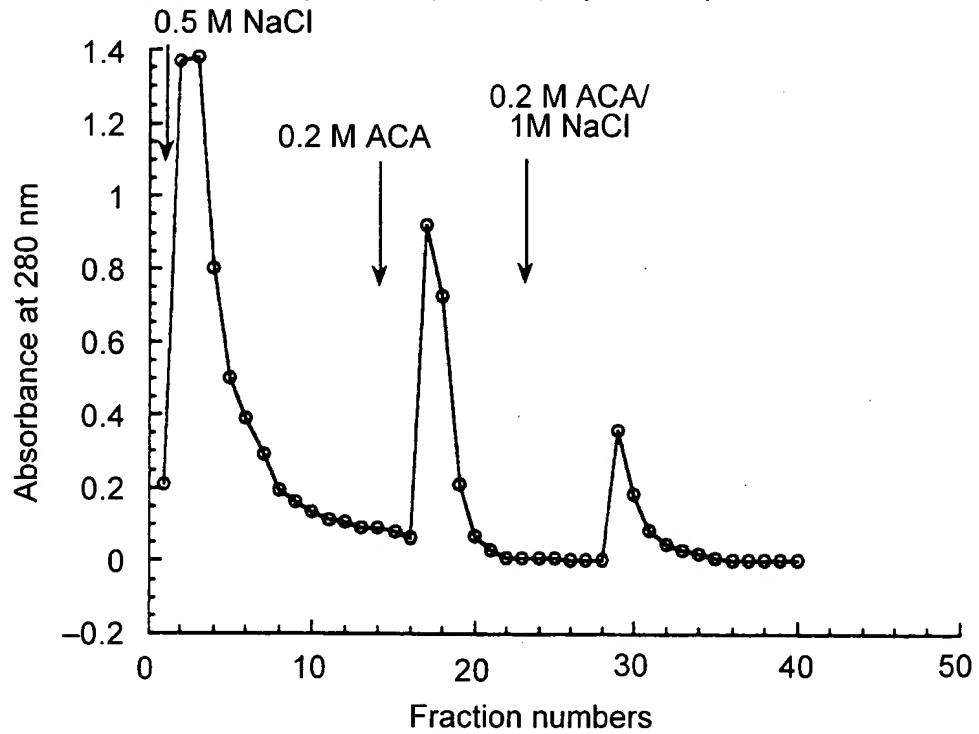


FIG. 21A

SDS-PAGE analysis of purified recombinant Angiostatin (Human):  
Lysine Sepharose column

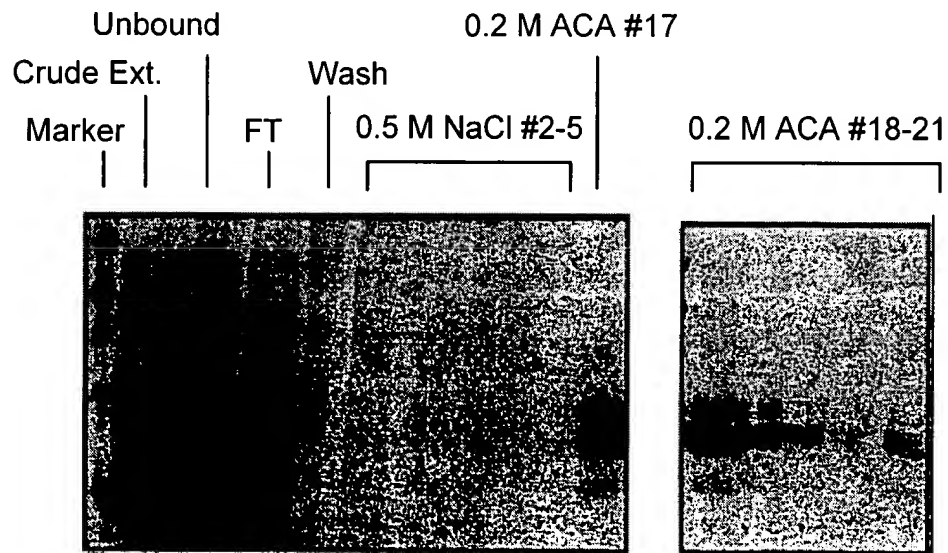


FIG. 21B





FIG. 22A
FIG. 22B

FIG. 22

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Sequence Range: 1-546  
Upstream primers for Restin and Apomigren are underlined,  
downstream primer for both is double underlined. Primer  
nucleotides not in the Restin sequence are shown in lower case.

```

ttt ttt gaa ttc->
  5   10   15   20   25   30   35   40   45
->ATT TCA AGT GCC AAT TAT GAG AAG CCT GCT CTG CAT TTG GCT GCT CTG
TAA AGT TCA CGG TTA ATA CTC TTC GGA CGA GAC GTA AAC CGA CGA GAC
50   55   60   65   70   75   80   85   90   95
AAC ATG CCA TTT TCT GGG GAC ATT CGA GCT GAT TTT CAG TGC TTC AAG
TTG TAC GGT AAA AGA CCC CTG TAA GCT CGA CTA AAA GTC ACG AAG TTC
100 105 110 115 120 125 130 135 140
CAG GCC AGA GCT GCA GGA CTG TTG TCC ACC TAC CGA GCA TTC TTA TCT
GTC CGG TCT CGA CGT CCT GAC AAC AGG TGG ATG GCT CGT AAG AAT AGA
145 150 155 160 165 170 175 180 185 190
TCC CAT TTG CAA GAT CTG TCC ACC ATT GTG AGG AAA GCA GAG AGA TAC
AGG GTA AAC GTT CTA GAC AGG TGG TAA CAC TCC TTT CGT CTC TCT ATG
195 200 205 210 215 220 225 230 235 240
AGC CTT CCC ATA GTG AAC CTC AAG GGC CAA GTA CTT TTT AAT AAT TGG
TCG GAA GGG TAT CAC TTG GAG TTC CCG GTT CAT GAA AAA TTA TTA ACC
```

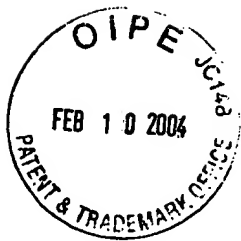
FIG. 22A



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245 250 255 260 265 270 275 280 285  
GAC TCA ATT TTT TCT GGC CAC GGA GGT CAG TTC AAT ATG CAT ATT CCA  
CTG AGT TAA AAA AGA CCG GTG CCT CCA GTC AAG TTA TAC GTA TAA GGT  
ttc cat atg->  
290 295 300 305 310 315 320 325 330 335  
->ATA TAC TCC TTT GAT GGT CGA GAC ATA ATG ACA GAT CCT TCT TGG CCC  
TAT ATG AGG AAA CTA CCA GCT CTG TAT TAC TGT CTA GGA AGA ACC GGG  
340 345 350 355 360 365 370 375 380  
CAG AAA GTC ATT TGG CAT GGC TCC AGC CCC CAT GGC GTC CGC CTT GTG  
GTC TTT CAG TAA ACC GTA CCG AGG TCG GGG GTA CCG CAG GCG GAA CAC  
385 390 395 400 405 410 415 420 425 430  
GAT AAC TAC TGT GAA GCA TGG CGA ACC GCG GAC ACA GCG GTC ACG GGA  
CTA TTG ATG ACA CTT CGT ACC GCT TGG CGC CTG TGT CGC CAG TGC CCT  
435 440 445 450 455 460 465 470 475 480  
CTT GCC TCC CCG CTG AGC ACG GGG AAG ATT CTG GAC CAG AAA GCA TAC  
GAA CCG AGG GGC GAC TCG TGC CCC TTC TAA GAC CTG GTC TTT CGT ATG  
485 490 495 500 505 510 515 520 525  
AGC TGT GCT AAT CGG CTA ATT GTC CTA TGT ATC GAA AAC AGT TTC ATG  
TCG ACA CGA TTA GCC GAT TAA CAG GAT ACA TAG CTT TTG TCA AAG TAC  
530 535 540 545  
ACA GAC GCT AGG AAG TAA  
TGT CTG CGA TCC TTC ATT cgc cgg cgt aag aa

FIG. 22B



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Sequence Range: 1 to 181

5	10	15	20	25	30	35	40	45
ISS ANY EKP ALH LAA LNM PFS GDI RAD FQC FKQ ARA AGL LST YRA FLS								
50	55	60	65	70	75	80	85	90
SHL QDL STI VRK AER YSL PIV NLK QOV LFN NWD SIF SGH GGQ FNM HIP								
100	105	110	115	120	125	130	135	140
IYS FDG RDI MTD PSW PQK VIW HGS SPH GVR LVD NYC EAW RTA DTA VTG								
145	150	155	160	165	170	175	180	
LAS PLS TGK ILD QKA YSC ANR LIV LCI ENS FMT DAR K								

FIG. 23

FLOW CHART: CLONING OF RESTIN (COIXV)  
INTO PICHIA EXPRESSION SYSTEM

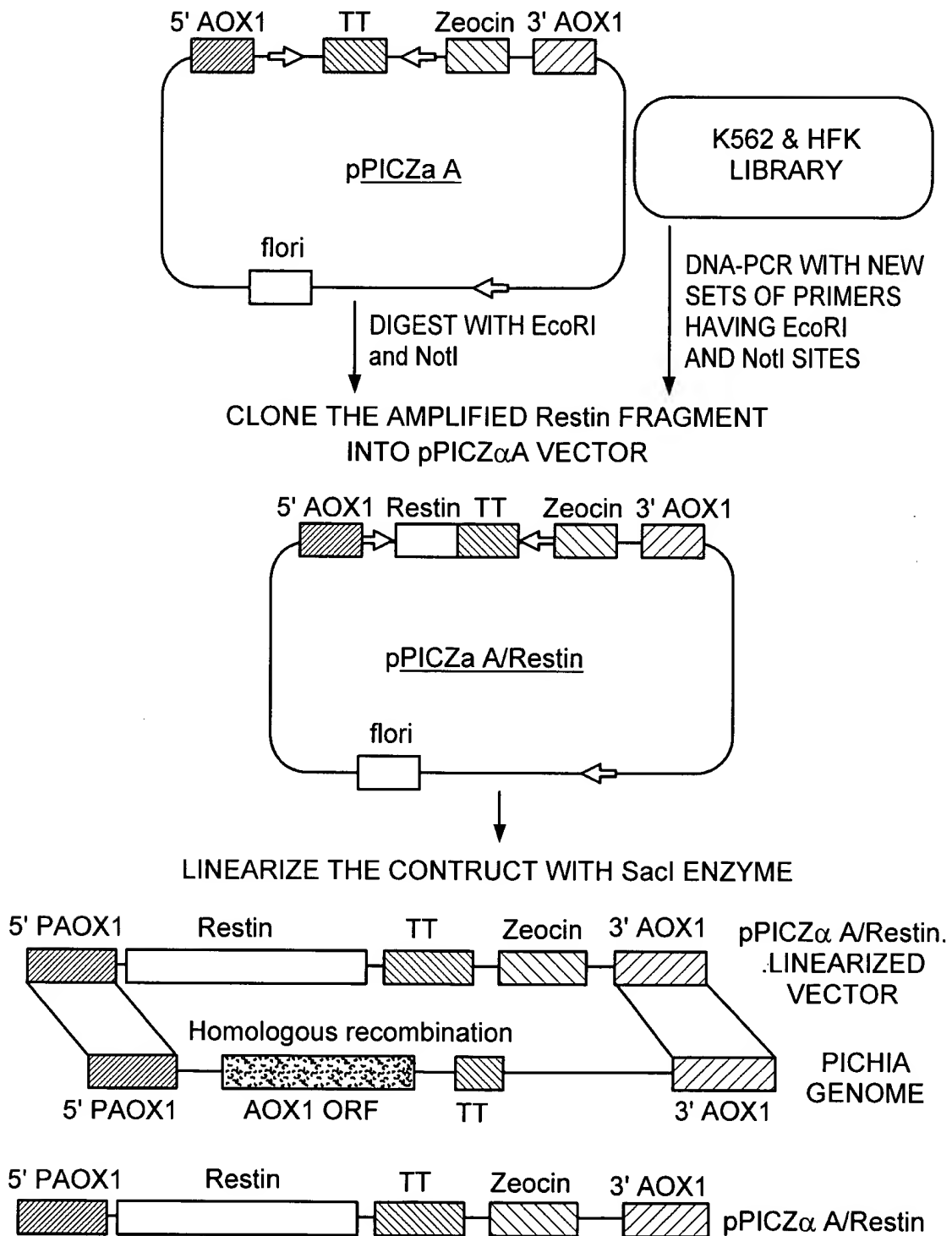


FIG. 24

# HUMAN RESTIN

Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
pPICZαA/ Restin	5' TTT TTT GAA TTC ATT TCA AGT- GCC AAT TAT GAG AAG CCT GCT- CTG CAT- TTG-3' (up) (SEQ ID NO:21)	EcoRI & NotI	Eukaryotic (Yeast), Pichia, pPICZαA  ( <i>yeast human restin</i> )	EF-restin (SEQ ID NO:27)
	5' AAG AAT GCG GCC GCT TAC TTC- CTA GCG TCT GTC ATG AAA CTG- TTT TCG AT-3' (down) (SEQ ID NO:22)			
pPICZαA/ His.Restin	5' AAT TCC ATC ACC ATC ACC ATC- ACG- 3' (up) (SEQ ID NO:23)	EcoRI (oligo insertion)	Eukaryotic (Yeast), Pichia, pPICZαA  ( <i>yeast human his.restin</i> )	EFHHHHHH-restin (SEQ ID NO:28)
	5' AAT TCG TGA TGG TGA TGG TGA- TGG-3' (down) (SEQ ID NO:24)			
pET28a/ apomigren	5' TTC CAT ATG ATA TAC TCC TTT- GAT GGT CGA GAC ATA ATG ACA-3' (up) (SEQ ID NO:25)	NdeI & NotI	Prokaryotic, pET system  ( <i>E.coli human apomigren</i> )	MGSSHHHHHHSSGLVPRGSHM-apo migren (SEQ ID NO:29)
	5' AAT GCG GCC GCT TAC TTC CTA- GCG TCT GTC ATG AAA CTG TTT- TCG AT-3' (down) (SEQ ID NO:26)			
pPICZαA/ apomigren	5' AAG AAT TCC ATC ATC ATC ATC- ATC ACA GCA GC-3' (up) (SEQ ID NO:11)	EcoRI & NotI	Eukaryotic (Yeast), Pichia, pPICZαA  ( <i>yeast human apomigren</i> )	EFMGSSHHHHHHSSGLVPRGSHM- apomigren (SEQ ID NO:30)
	5' AAT GCG GCC GCT TAC TTC CTA- GCG TCT GTC ATG AAA CTG TTT- TCG AT-3' (down) (SEQ ID NO:26)			

FIG. 25

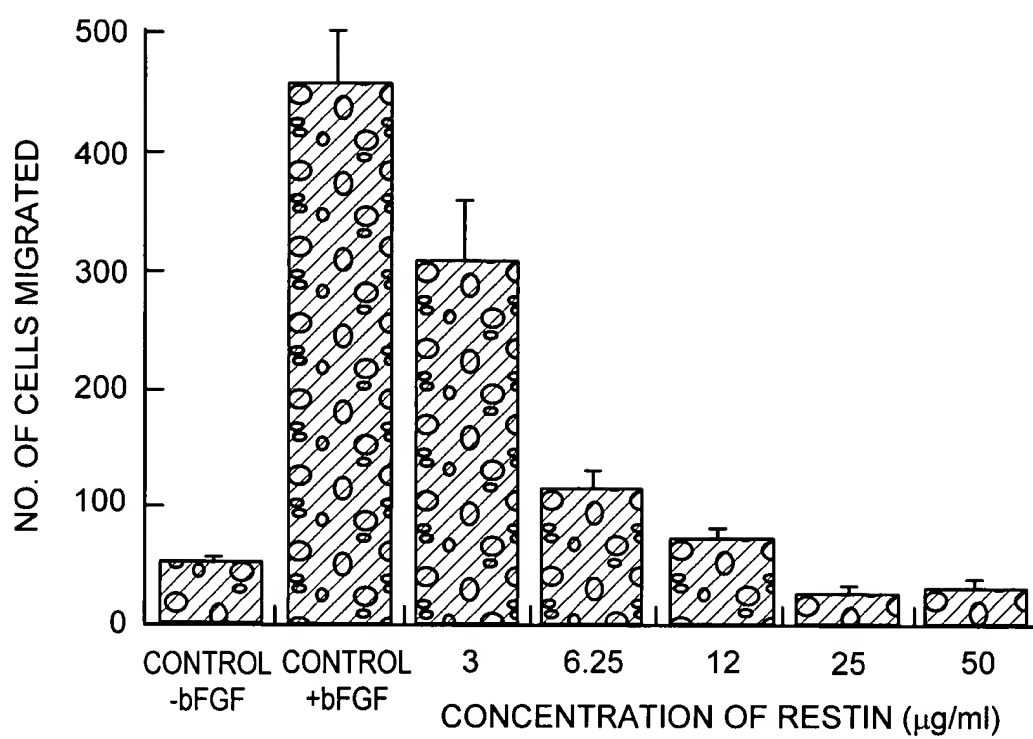


FIG. 26

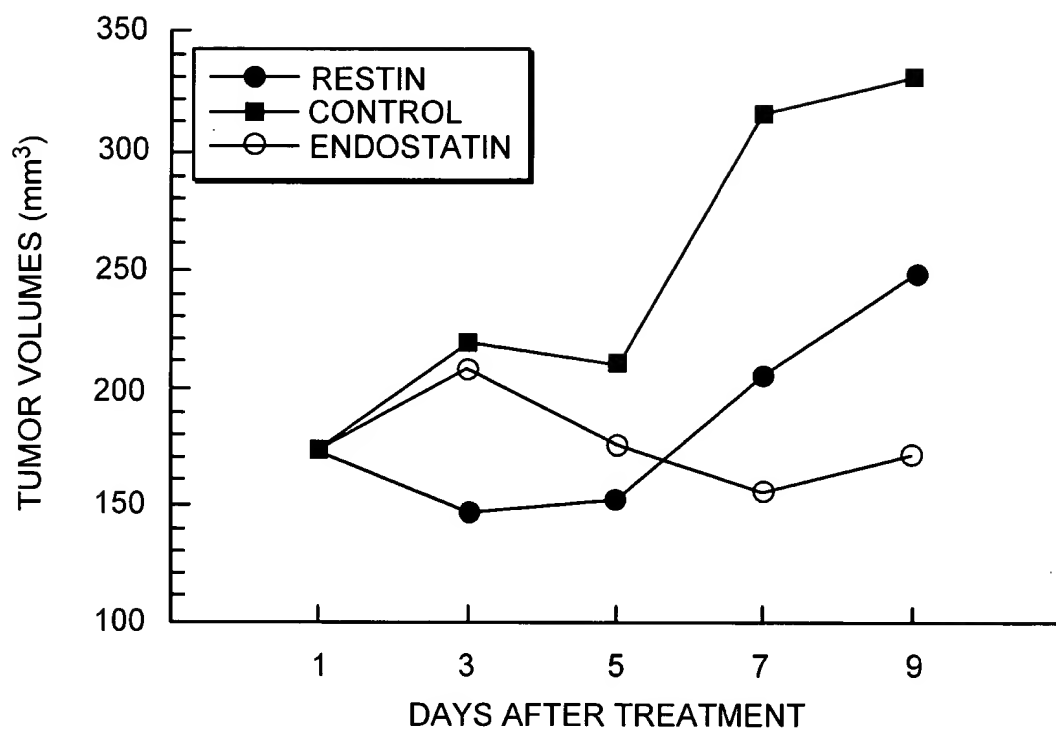


FIG. 27